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AN ANALYSIS OF THE RELATION BETWEEN GROWTH AND NUCLEAR DIVISION IN A PARASITIC INFUSORION, OPALINA SP.[1]

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THIS investigation was undertaken for the purpose of analyzing the relation between growth and nuclear division in a species of *Opalina* of the frog during the growth period in the tadpole. The multinucleate condition of *Opalina* and the absence of cell walls render it of particular value as material for the study of the phenomena involved in nuclear division and growth. The specimens used in our investigations were obtained by Dr. Charles E. Simon from tadpoles collected at Chester, Nova Scotia, during the summer of 1920. Unfortunately we are unable to state either the species name of the *Opalina* or that of the host. Dr. Maynard M. Metcalf, who has examined the slides, thinks the *Opalina* is probably an undescribed species. The material was well fixed in Schaudinn's solution and beautifully stained with iron-hematoxylin.

A sufficiently large number of specimens (455) were drawn with a camera lucida so as to furnish reliable results when measurements were treated by statistical methods. The area of the drawings was determined with a planimeter and the correlation with the nuclear number determined. Table I is the correlation table for the nuclear number and area of 341 specimens. The area of the drawings, which were made at a magnification of 650 diameters, is given in square millimeters. The coefficient of correlation is remarkably high, namely, .755 ± .016; this proves that an increase in size is accompanied by a corresponding increase in nuclear number. The rest of the specimens that were measured, 114 in number, were drawn at a magnification of 1400 diameters. The coefficient of correlation of this lot was found to be .875 ± .015.

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[1] From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

TABLE I

CORRELATION TABLE FOR NUMBER OF NUCLEI AND AREA OF 341 SPECIMENS

The area is given in sq. mm. and obtained from camera-lucida drawings made at a magnification of 650 diameters. Coefficient of correlation .755 ± .016.

Area	Number of Nuclei																
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	27	28	29	
300+		1																1
400+	1	1	2				1											5
500+		1	1	1	1													4
600+	1	7	7	3	1													19
700+		4	6	12	2													24
800+		2	12	13	5	3		1										36
900+			6	25	8	6	1											46
1000+			1	13	14	9	8											45
1100+		2		3	12	20	6	4	1									48
1200+			1	1	8	3	5	5	1									24
1300+	1	1	1	1	1	5	4	4	1		2							21
1400+				1	1	3	4	5	4	2								20
1500+				1				4	1	1	1	3						11
1600+							1	1	3	2	1		1					9
1700+			1		1		1	1							1			4
1800+			1						2	2					1			6
1900+			1	1		1	1		1	1	1							7
2000+													1					1
2100+									1	1		2						4
2200+									1									1
2400+								1					1	1				3
2800+																	1	1
3200+																1		1
	3	19	40	75	54	50	32	26	16	9	5	5	3	1	1	1	1	341

Metcalf[2] has pointed out that in multinucleate Opalinas the nuclei within a single specimen may be in different stages of division at one time. This we have found to be true also of the nuclei during the growth stages in the tadpole—a condition that has enabled us to analyze with considerable accuracy the exact relation between cytoplasmic mass and nuclear division. For example, among the specimens with four nuclei, there were a few with three "resting" nuclei and one nucleus in division (Fig. 3); obviously one of the four nuclei is undergoing division before its three sisters. If the sum of the areas of a number of specimens in which there are four nuclei of equal size (Fig. 2) is divided by the total number of nuclei, a fairly accurate idea may be obtained of the amount of cytoplasm associated with each nucleus. According to the nucleo-cytoplasmic relation theory[3] an increase in the amount of cytoplasm as compared with the amount of nuclear material furnishes the stimulus which initiates nuclear division. A comparison between specimens with four equal nuclei, and specimens with four nuclei one of which is undergoing division, should reveal approximately the increase of cytoplasmic substance necessary to inaugurate nuclear division. A number of cases of this sort were available in our material and were studied with the following results.

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[2] Metcalf, M. M., 1909, "*Opalina*," *Arch. f. Protist.*, 13: 195–375. Especially p. 269.

Table II shows the relations between area, and number, volume and surface of the nuclei in the 207 specimens that could be used for this purpose. The measurements were made of camera-lucida drawings at a magnification of 650 diameters. Beginning with the group of 15 at the top of the table we can make the following comparisons.

1. Fifteen specimens, each with 4 equal nuclei (Fig. 2), have an average area per nucleus of 176.1 sq. mm.; 10 specimens, each with 3 equal nuclei and a fourth nucleus in division (Fig. 3), have an average area per nucleus of 185.0 sq. mm. The specimens in which division has been initiated have an average area per nucleus 8.9 sq. mm. greater than those with an equal number of nuclei, none of which are in division. We have used the area through-

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[3] For a recent discussion of this theory see Hegner, R. W. 1920, "Relations between Nuclear Number, Chromatin Mass, Cytoplasmic Mass, and Shell Characteristics in Four Species of the Genus *Arcella*," *Jour. Exp. Zool.*, 30: 1–95.

TABLE II

Table Showing the Relation between Area and Number, Volume and Surface of the Nuclei

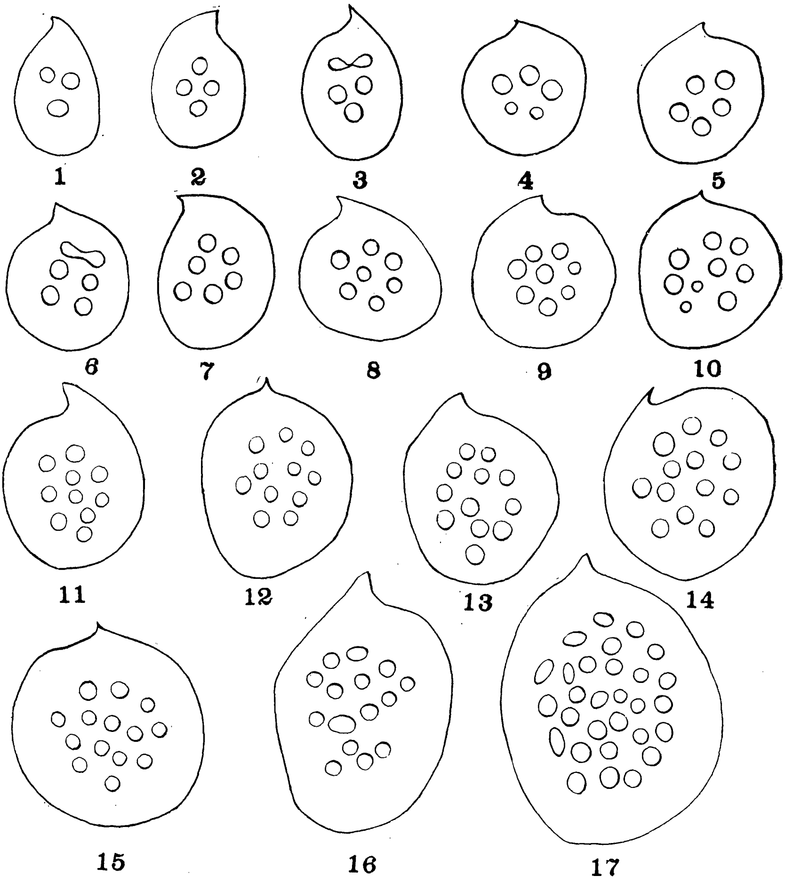
The area, volume and surface of the nuclei were computed from camera drawings made at a magnification of 650 diameters.

Number of Specimens	Number of Nuclei	Average Area in Sq. Mm. at Magnification of 650 Diameters	Average Area per Nucleus in Sq. Mm. at Magnification of 650 Diameters	Average Volume of Nuclei in Cubic Mm. at Magnification of 650 Diameters	Average Volume per Nucleus in Cubic Mm. at Magnification of 650 Diameters	Average Surface of Nuclei in Sq. Mm. at Magnification of 650 Diameters	Average Surface per Nucleus in Sq. Mm. at Magnification of 650 Diameters
15	4 (equal in size)	704.2	176.1	215.49	53.87	261.32	65.33
10...	4 (one in division)	739.9	185.0				
13...	5 (two small)	851.5	170.3	257.11	51.42	321.10	64.22
16...	5 (equal in size)	860.5	172.1	281.91	56.38	345.01	69.00
17...	6 (two small)	951.4	158.6	273.69	45.62	363.85	60.64
49...	6 (equal in size)	926.7	154.5	264.83	44.14	352.89	58.81
5...	6 (one in division)	1,032.5	172.1				
11...	7 (two small)	993.5	141.9				
31...	7 (equal in size)	1,066.7	152.4	269.17	38.45	379.68	54.24
10...	7 (one in division)	1,176.7	168.1				
30...	8 equal	1,127.9	141.0	336.53	42.07	458.93	57.37
207							

out our work as a measure of cytoplasmic mass, hence it appears from the results of our measurements that an increase in mass per nucleus represented by an increase in area within the limits of 8.9 sq. mm. is the stimulus that initiates nuclear division. The exact mass of cytoplasm represented by this increase in area of 8.9 sq. mm. might easily be obtained under more favorable circumstances.

2. When we compare the measurements of the 10 specimens with 4 nuclei, one of which is dividing (Fig. 3) with 13 specimens of the stage immediately following, with 3 large nuclei and two that have just reorganized after division (Fig. 4), we find that although the latter average 111.6 sq. mm. larger per specimen the average area per nucleus is 14.7 sq. mm. less. Thus there has been an

actual increase in size but a decrease in the mass of cytoplasm associated with each nucleus.



FIGS. 1–17. Outline drawings of stages in the growth of *Opalina* sp. made with a camera lucida at a magnification of 650 diameters and reduced to a magnification of 325 diameters.

3. During the growth of the two small nuclei to their full size (Figs. 4–5) the size of the organism increases from an average area of 851.5 sq. mm. per specimen to an average area of 860.5 sq. mm., or an average area per nucleus of from 170.3 sq. mm. to 172.1 sq. mm. Although an increase in size has taken place, the average area per nucleus of 172.1 sq. mm. in specimens containing 5 full-

sized nuclei is less than that of specimens with 4 full-sized nuclei, *i.e.,* 176.1 sq. mm.

4. A further increase in the average size of the specimens occurs between the stage with 5 nuclei of equal size (Fig. 5) and that with 6 nuclei, two of which have just emerged from mitosis. Measurements give for the former an average area of 860.5 sq. mm. and for the latter 951.4 sq. mm. The specimens with the two small nuclei, however, possess as before (see (2)) a lower average area per nucleus, *i.e.,* 158.6 sq. mm. as compared with 172.1 sq. mm. in specimens with 5 full-grown nuclei.

5. The measurements of the next stage, *i.e.,* specimens with 6 nuclei of equal size (Fig. 7), are more difficult to explain, since the average area of the specimens (926.7 sq. mm.) is actually less than that of the younger specimens (see (4)) with 4 large and 2 small nuclei, and the average area per nucleus falls from 158.6 sq. mm. to 154.5 sq. mm. These results may be due to a thickening of the entire animal which would increase the mass and tend toward a decrease in area or the nucleo-cytoplasmic relation may change as the animals become older. That there is an actual decrease in the average area per nucleus as growth proceeds is indicated by the measurements of later stages as given in Table III. This table shows a decrease per nuclear area from 186 sq. mm. in specimens with 4 nuclei to 96.8 sq. mm. in specimens with 29 nuclei. That this decrease is gradual is indicated when averages are made of three successive groups containing each a larger number of specimens. Thus the 4, 5, and 6 nucleated groups containing 134 specimens have an average area per nucleus of 173.8 sq. mm., the 7, 8, and 9 nucleated groups containing 136 specimens have an average area per nucleus of 144.4 sq. mm., the 10, 11, and 12 nucleated groups containing 51 specimens have an average area per nucleus of 143.5 sq. mm., and the 13, 14, and 15 nucleated groups containing 13 specimens have an average area per nucleus of 128.3 sq. mm.

TABLE III

TABLE GIVING THE AVERAGE AREA PER SPECIMEN AND PER NUCLEUS OF 338 SPECIMENS DRAWN WITH A CAMERA LUCIDA AT A MAGNIFICATION OF 650 DIAMETERS

Number of Specimens.	Number of Nuclei.	Average Area per Specimen in Sq. Mm. at Magnification of 650 Diameters.	Average Area per Nucleus in Sq. Mm. at Magnification of 650 Diameters.
19......	4	744	186.0
40......	5	891	178.2
75......	6	944	157.3
54......	7	1,069	152.7
50......	8	1,150	143.7
32......	9	1,231	136.8
26......	10	1,395	139.5
16......	11	1,635	148.6
9......	12	1,709	142.4
5......	13	1,572	120.9
5......	14	1,801	128.6
3......	15	2,032	135.4
1......	16	2,452	153.2
1......	17	1,806	106.2
1......	28	3,277	117.0
1......	29	2,806	96.8

6. By the time one of the nuclei of the six-nucleated stage has been stimulated to division the average area per nucleus increases again to 172.1 sq. mm. After this division is completed and seven nuclei are present, two of them small, the average area per nucleus, as was to be expected, decreased to 141.9 sq. mm. During the period necessary for the two small nuclei to reach their full size (Fig. 8) the area increases again to 152.4 sq. mm. A further increase to 168.1 sq. mm. occurs by the time sufficient growth takes place to stimulate one of these seven nuclei to divide, and a decrease (to 141.0 sq. mm.) again takes place when this stage evolves into that with eight nuclei (Fig. 9).

The two curves in Fig. 18 show clearly the increase in area per nucleus up to the point where one nucleus divides, then a conspicuous decrease following nuclear division, and subsequently an increase during the period when the nuclei resulting from division regain their full size, ending in a size at which the area per nucleus is approximately that present at the beginning.

Figure 19 illustrates the fact that the size of the entire specimens increases during nuclear multiplication and growth, but that the area per nucleus remains almost constant.

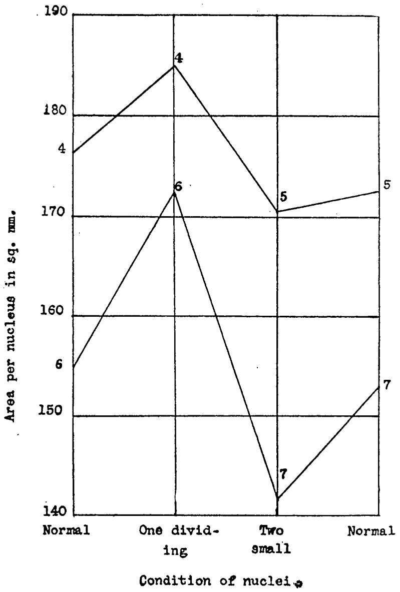


FIG. 18. Curves showing the changes in area per nucleus that accompany changes in nuclear number and condition. The numbers 4, 5, 6, and 7 indicate the number of nuclei present.

7. The series of measurements of these specimens affords an explanation of the reason why nuclear division in *Opalina* is not synchronous. According to the nucleo-cytoplasmic relation theory, as soon as the mass of cytoplasm has increased to a certain point nuclear division is initiated. The necessary increase to furnish this stimulus in *Opalina* may be determined approximately from our data by comparing measurements of specimens in which the nuclei are all equal in size with those in which nuclear division has been inaugurated. Such a comparison gives the following results.

Nuclei	Average Area per Nucleus	Difference in Area — Amount Necessary to Stimulate Division
4 (equal)	176.1 sq. mm.	
4 (one in division)	185.0 sq. mm.	8.9 sq. mm.
6 (equal)	154.5 sq. mm.	
6 (one in division)	172.1 sq. mm.	17.6 sq. mm.
7 (equal)	152.4 sq. mm.	
7 (one in division)	168.1 sq. mm.	15.7 sq. mm.

These figures, of course, indicate only the relative increase necessary to stimulate nuclear division; the actual increase could be determined by measuring accurately the mass of cytoplasm in each case.

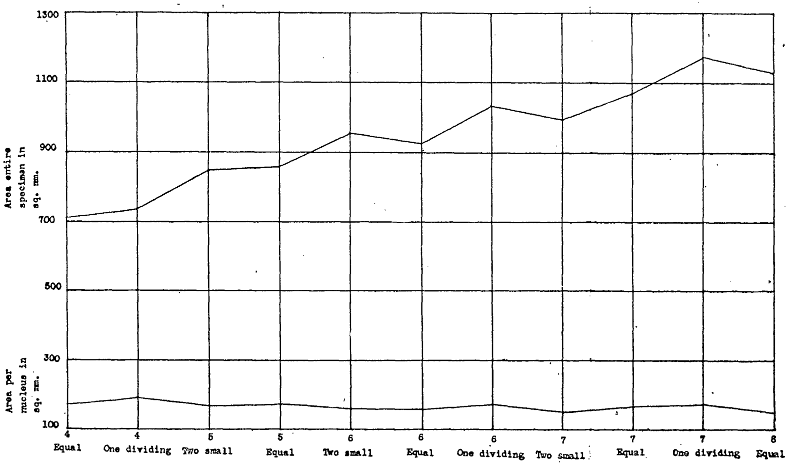


FIG. 19. Curves showing the increase in the area of specimens with increase in age and the constancy of the area per nucleus.

In looking over our camera drawings it was noticed that usually only one nucleus was in division in any one specimen (Figs. 3 and 6) and that in many cases two of the nuclei in a specimen were smaller than the rest (Fig. 4), indicating that they were daughter nuclei that had just emerged from mitosis. Of a total of 137 specimens in which nuclei were found in division, 109 contained one division figure, 19 contained 2, 8 contained 3, and 1 contained 4. Furthermore, those containing more than one division figure were usually older than those containing one only. Thus the average nuclear number of specimens with one dividing nucleus was 7.2, with two dividing nuclei, 8.1, with three dividing nuclei, 9.3, and with 4 dividing nuclei, 10. These data favor the conclusion that the stimulus that initiates nuclear division acts as a rule on only one nucleus at a time and that the division of this nucleus restores the nucleo-cytoplasmic ratio. When this ratio is again disturbed by an increase of the cytoplasmic mass another nucleus is stimulated to divide. Division of two or more nuclei synchronously may be due to the more rapid growth, the larger specimens in which this usually occurs, or to the greater chances of two or more nuclei reacting to the division-stimulus when a large number of nuclei are present in a single specimen. There is some evidence that the nucleus that undergoes division is the one with the greatest amount of cytoplasm surrounding it, but this could not be determined definitely. No regular distribution of the nuclei was evident. It is interesting to note in this connection that during the embryonic development of many animals nuclear division occurs in all cells at nearly the same time. This is especially interesting in the case of certain insects, in the eggs of which nuclear division proceeds synchronously without the intervention of cell walls until thousands of nuclei are present in a single egg.[4] An increase of cytoplasm over nucleus may also, in these insect eggs, stimulate nuclear division, since after each division the mass of cytoplasm surrounding each nucleus is increased by the addition of new material elaborated from the yolk substance in which it is situated.

8. The average total volume of the nuclei of certain specimens, average volume per nucleus, average total area of the surface of the nuclei and average area of the surface per nucleus were measured in cubic millimeters and square millimeters from our camera drawings which

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[4] Hegner, R. W., 1914, "Studies on Germ Cells," *Jour. Morph.*, 25: 375–509. Especially pp. 408–413.

were magnified 650 diameters. These data are shown in Table II. They indicate one point of considerable interest and that is the fact that as the number of nuclei increases their average volume and surface decrease.

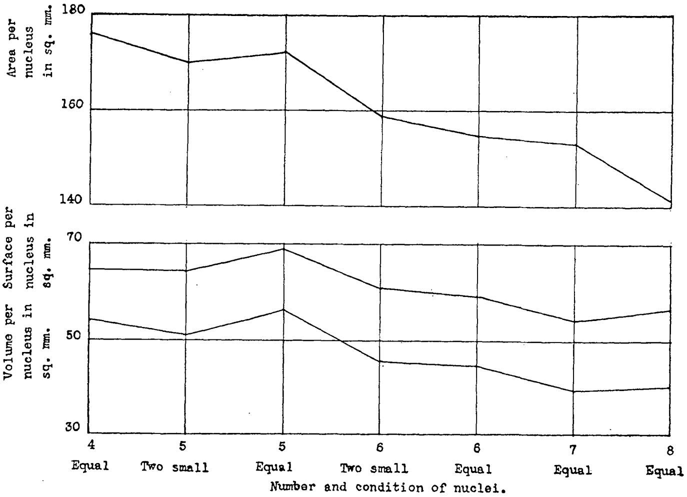

FIG. 20. These three curves show that decreases in the volume and surface of the nuclei are accompanied by decreases in area per nucleus.

Thus in specimens with 4 nuclei of equal size each nucleus has an average volume of 53.87 cu. mm., in specimens with 6 nuclei the average volume per nucleus decreases to 44.14 cu. mm., and in specimens with 8 nuclei the average volume per nucleus decreases still further to 42.07 cu. mm. Similar results were obtained from measurements of the surface of the nuclei, but the decrease is not so great since the volume decreases as the cube whereas the surface decreases only as the square. As the table (II) shows the average surface per nucleus in sq. mm. decreased from 65.33 sq. mm. in specimens with 4 nuclei, to 58.81 sq. mm. in specimens with 6 nuclei, and 57.37 sq. mm. in specimens with 8 nuclei. This decrease in volume and surface may account for the fact noted previously (in (5)), that the area per nucleus decreases in specimens with nuclei all equal in size as the number of nuclei becomes greater with advancing age. Since the volume of the nuclei is less in these older specimens the amount of cytoplasm associated in normal nucleo-cytoplasmic relations with them is less and the area of the specimens per nucleus decreases accordingly.

The curves in Fig. 20 bring out clearly the relation between area and volume and surface of the nuclei during the growth period. The average area per nucleus decreases as the number of nuclei increases, but at the same time there is a corresponding decrease in both volume and surface of the nuclei, thus maintaining approximately the initial relation between nucleus and cytoplasm.

SUMMARY

(*a*) A high correlation exists between nuclear number and cytoplasmic mass (as indicated by area) during the growth of *Opalina* sp. The coefficient of correlation in one lot of 341 specimens was .755 ± .016 and in another lot of 144 specimens was .874 ± .015.

(*b*) By comparing the area of various stages with the number, size, state of division, volume and surface of the nuclei the following conclusions were reached. (1) Nuclear division is stimulated by an increase of cytoplasm that may be determined approximately. (2) As the organisms increase in age the nuclei decrease in volume and surface; this is accompanied by a corresponding decrease in the area per nucleus, indicating that the nucleo-cytoplasmic relation is maintained. (3) Nuclear division is not synchronous because one nucleus is usually stimulated to divide before the others, and this division is sufficient for the time to reestablish the normal relation between nuclei and cytoplasm.